

Application No.: 09/027,654

Docket No.: 28911/34561

AMENDMENTS TO THE CLAIMS

Please amend the claims by canceling claim 19 in the following manner. This listing of claims will replace all prior versions, and listings, of claims in the application.

1. (Previously presented) An improved method of conducting a specific binding assay for the presence of an intracellular analyte in a cultured cell sample which method comprises the steps of:

- i) mixing a sample of cultured cells with a cell lysis reagent to provide a lysed cellular sample;
- ii) mixing and reacting the lysed cellular sample with a specific binding assay reagent comprising a specific binding partner of the intracellular analyte and a tracer to perform a specific binding assay; thus forming a reaction mixture comprising a specific-binding partner-intracellular analyte complex;
- iii) mixing the lysed cellular sample with a cyclodextrin sequestrant for the cell lysis reagent, whereby the specific binding assay of step ii) is performed in the presence of the sequestrant; and
- iv) detecting the presence of the specific binding partner-intracellular

analyte complex, the presence of which is indicative of the presence of intracellular analyte in the sample wherein the improvement lies in the sequestrant *adding in the amount of 0.5 - 10% by weight based on the weight of the reaction mixture.*

~~from and directly affecting a binding reaction between the analyte and its specific binding~~

~~particulate~~

2. (Previously presented) The method as claimed in claim 1, wherein the cell lysis reagent is a detergent.

3. (Cancelled)

Application No.: 09/027,654

Docket No.: 28911/34561

3 ✓ (Previously presented) The method as claimed in claim 1, wherein the amount of cyclodextrin sequestrant is in the range of 1 - 5% of the said reaction mixture.

4 ✓ (Original) A method as claimed in claim 1, wherein steps i), ii) and iii) are all performed in a single reaction vessel.

5 ✓ (Original) A method as claimed in claim 1, wherein multiple assays are performed in parallel in wells of a multiwell plate.

6 ✓ (Original) A method as claimed in claim 1, wherein the cells are cultured in a vessel and are lysed in that vessel for assaying the analyte in that vessel.

7 ✓ (Original) A method as claimed in claim 1, wherein the assay of step ii) is a homogenous assay.

8 ✓ (Original) A method as claimed in claim 1, wherein the assay of step ii) is a homogenous assay.

9 ✓ (Original) A method as claimed in claim 1, wherein the specific binding assay of step ii) is an immunoassay.

10 ✓ (Original) A method as claimed in claim 1, wherein the analyte is adenosine-3', 5'-cyclic monophosphate, the cell lysis reagent is dodecyl trimethyl ammonium bromide and the sequestrant is α -cyclodextrin.

Application No.: 09/027,654

Docket No.: 28911/34561

11 ~~12.~~ (Original) A method as claimed in claim 1, wherein the cells have been maintained in a culture medium, and step i) is performed in the presence of the culture medium.

12 ~~12.~~ (Original) A method as claimed in claim 1, wherein the intracellular or ~~the total~~ intracellular plus extracellular concentration is measured of an analyte selected from adenosine-3', 5'-cyclic monophosphate, interleukin-6 and prostaglandin E₂.

14. (Previously presented) A kit, suitable for assaying for an analyte by the method as claimed in claim 17 comprising: a detergent; a sequestrant for the detergent; a specific binding partner of the analyte; a tracer; and separation means for separating bound tracer from unbound tracer.

15. (Cancelled)

13 ~~16.~~ (Original) The method as claimed in claim 1, wherein the specific binding assay is a receptor binding assay.

14 ~~17.~~ (Previously presented) The method as claimed in claim 1, which further comprises the step of separating bound tracer from unbound tracer.

Application No.: 09/027,654

Docket No.: 28911/34561

15 ~~18.~~ (Previously presented) The method as claimed in claim 1, wherein the tracer is selected from the group consisting of radioactive isotope label, enzyme-linked label and fluorescent label.

19. (Cancelled)

GI 20. ~~(Previously presented) A kit suitable for assaying for an analyte by the method claimed in Claim 1, comprising a detergent, a cyclodextrin sequestrant for the detergent, a specific binding partner for the analyte and a vessel suitable for cell culture.~~

16 ~~21.~~ (Original) A method as claimed in claim 1 wherein the specific binding assay of step ii) is a fluorescence polarization immunoassay.
